SUMMARIES IN MICRO-BIOLOGY

For some months the Secretary has been planning to secure for this Journal and its Department of Summaries, a series of papers from biologists dealing with the chief groups of microscopic plants and animals. It has not been the purpose to present a complete survey of any of the groups. The wish has been rather to bring together in one article a statement of the following things:—general biology, the method of finding, the methods of capture and of keeping alive and cultivating in the laboratory; how best to study; the general technic; the most accessible literature; and a brief outline of the classification, with keys for the identification of at least the more representative genera and species of the micro-organisms likely to be found by the beginning students in the United States.

It has been felt that the getting together of such data as this, while not a contribution to science, would be a contribution especially to isolated workers and to teachers and stu-

dents in the high schools and smaller colleges.

Papers have already appeared treating the aquatic Oligochetes, the Melanconiales and the Rusts. The following is the fourth paper of the series. It is proposed to have such synopses from time to time until the more common American species of such groups as the following have been covered: The Blue-green Algae, Conjugating Algae, Diatoms, other Green Algae, Downy Mildews, Yeasts, Powdery Mildews, Hyphomycetes, Smuts. Rhizopods, Infusoria, Turbelaria, Bryzoa, Water Mites, Entomostraca, etc.—[Editor.]

THE BLACK MOULDS (Mucoraceae) LEVA B. WALKER

The Mucoraceae receive the name "Black Moulds" from the fact that in a number of the most conspicuous genera the fruiting bodies and older hyphae are dark colored or "black." A young growth of any of these fungi is, however, always colorless. They are largely saprophytic and are found abundantly on decaying organic matter, especially on dung, and in the soil as common soil fungi. The parasitic forms live upon other mucors or upon basidiomycetes. Almost all are easily cultivated on bread, on dung or on ordinary culture media (either directly or by culturing the host and parasite together) if bateriological apparatus is at hand.

1. Plant Body

The Mucoraceae form a well-defined group. The plant body, as is true for almost all phycomycetous fungi, is siphonaceous, cross walls being few or none, always coenocytic. The protoplasm in young growing filaments shows characteristic movement in a "glacier-like" fashion. The mycelium may be superficial, developing rhizoids that penetrate the substratum, or it may develop almost entirely within the substratum.

2. Sexual Reproduction

The most uniform character in the Mucoraceae is the production of zygospores. The zygospores are developed by the union of cells from the same filament or from other filaments. First very much enlarged branches (progametes) branch off from two adjacent filaments (Plate IV, Fig. 1, a), a cell is cut off from the end of each progamete, these end cells becoming the gametes (Fig. 1, b (x)), the enlarged supporting cell being called the suspensor (Fig. 1, b (y)). The gametes fuse (Fig. 1, c) by the absorption of the wall between the gametes, and a thick wall is built up around the fertilized cell, which is then called the zygospore. While zygospores are known for most of the species of the Mucoraceae, they are rarely found. When they do appear in cultures they appear in great abundance. The conditions necessary for the production of zygospores has been the subject of many researches, the most extensive of which are those of Blakeslee, who has shown definitely that the Mucors are separable into two distinct types which he terms homothallic and heterothallic. In homothallic forms the gametes are produced from branches of the same filament and zygospores are usually abundant in nature wherever the fungus is found. In the heterothallic forms the gametes must be produced from separate strains (termed for convenience + and —) of the fungus which often differ so greatly in appearance that they might easily be taken for separate species or at least varieties. Extensive experiments made by planting + and — strains of many Mucors together show that when a + and a - of different species are planted together the progametes will form (but will not form zygospores), but that when a + and a + or a - and a - are planted together no such progametes are formed. The + strain is usually a little more vigorous than the -, and in some cases the gametes are larger, so we can well think of the + strain as female and the — as male. In heterothallic forms the zvgospores are rarely found, as either the + or the — is rare. The germination of the zygospores has been observed in relatively few cases, but where observed takes place much as shown in Fig. 3, 1. Zygospores germinate only after a resting period.

3. Asexual Reproduction

Sporangiospores—The sporangiospores (usually referred to

simply as spores) are typically formed in sporangia which are produced on erect well-differentiated hyphae known as sporangiophores. The sporangia are separated from the sporangiophores by a cross partition which usually rounds up into the sporangium, forming a columella (Fig. 1, f). The outer wall of the sporangium is usually delicate, dissolving in water, or fracturing easily so that in examining material in water mounts under the microscope the spores are often seen surrounding the columella, the only remnants of the outer wall being seen at the point of its attachment to the columella. The sporangia are in most cases evident and many spored, but in one tribe they are reduced seemingly to one spored sporangia and the one celled sporangia are usualy called "conidia." In another tribe the spores are arranged in a single row in a sporangium which breaks down so readily that it is rarely distinguishable and gives the appearance of a chain of "conidia."

The asexual reproduction as above described is always the most abundant form of reproduction and some forms are only known by their asexual reproduction. For this reason the keys which will follow are based upon the sporangial reproduction. The spores are capable of germination at once (Fig. 3, above i), but will usually remain viable for an indefinite period.

AZYGOSPORES—These are often found in cultures of Mucors, they usually appear much like the zygospores, but are formed without the union of gametes.

CHLAMYDOSPORES—Thick-walled spores, known as chlamydospores, are often found in the main vegetative filament, or on the ends of filaments (Fig. 3, i, k). They may germinate at once or after a resting period.

4. Systematic

The Mucoraceae comprise a group of about forty described genera and over three hundred described species. Probably most of these are present in our flora. Only a few of the most typical and abundant genera and species will be mentioned here.

KEY TO THE TRIBES OF MUCORACEAE.

- A. Reproduction asexually by spores contained in evident sporangia.
 - I. Columella present.
 - a. Sporangia many-spored, generally of one kind or if two kinds the smaller irregularly disposed on the main sporangiophore.
- B. Reproduction asexually by so-called "conidia" produced either solitary or in chains.

 - II. "Conidia" in chains, produced in head-like masses upon sterigmata at the ends of "conidiophores"............Cephalideae (VI)

I. TRIBE MUCOREAE

Sporangia generally of one kind with a columella and a membrane that dissolves or fractures easily. Smaller sporangia (sporangioles) with persistent membranes occur very rarely and in such cases are disposed without order upon the main sporangiophore. Zygospores naked or surrounded by appendages.

KEY TO GENERA.

- I. Sporangiophores fasciculate on a rhizoidiferous stolon.
 - I. Sporangia globose; zygospores unprotected......Rhizopus
- II. Sporangiophores emerging solitary from the mycelium, no rhizoidiferous stolons.
 - I. Sporangiophores unbranched, or not dichotomously branched.
 - a. Mycelium of one kind.
 - 1. Zygospores unprotected.
 - a. Gametes about equal (heterothallic or produced from widely separated parts of the mycelium)......Mucor

RHIZOPUS (=Ascophora)—Saprophytic fungi, the hyphae non-septate and much branched, forming long stolons and rhizoids. Sporangiophores clustered at the nodes (Fig. 1, e) above the rhizoids. Sporangia spherical containing many spores. Zygospores spherical or nearly so with a thick, warty dark-brown wall: heterothallic.

R. nigricans Ehrb. The stolons far spreading, often 1-4 cm. long, covering the substratum with a cobwebby growth which is at first colorless, but finally brown. Rhizoids much branched, colorless at first, finally becoming brown. Sporangiophores mostly in clusters of 3-5 (seldom single), unbranched 0.5-4 mm. high. Sporangium and columella (Fig. 1, f). Sporangia 100-350 μ in diameter. Spores irregularly globose or broad oval 6-17 μ with longitudinal ridges, light gray (Fig. 1, g). Zygospores 160-220 μ in diameter, brownblack, opaque, warty (Fig. 1, d).

On all kinds of organic matter. It is our most common black mould and will usually appear in a few days upon moist bread or any organic substance when placed in a moist chamber. Cultures can always be easily obtained by breaking open a sweet potato that has rotted with a soft rot and placing it in a moist chamber for a few days. The rot is caused by the fungus. It also causes large proportion of the rot of strawberries. In growth upon these hosts the stoloniferous habit is often not seen.

ABSIDIA—Saprophytic fungi. Mycelium stoloniferous; sporangiophores in groups produced only on the tips of the arched internodes (Fig. 2, a). Sporangia pyriform (Fig. 2, c). Columella blue-black. Zygospores protected by flexuous circinate outgrowths from one or both suspensors (Fig. 2, b). Both heterothallic and homothallic forms known.

A. caerulea Bainier. Vegetative hyphae blue-violet, sporangiophores up to 25 mm. in length. Sporangia pale-violet to brown bearing many pale-violet spores 4-7 μ . Columella hemispherical or abconical, often surmounted by a nipple (Fig. 2, d). Zygospores 60μ in diameter with suspensors provided with 10-20 long slender circinate appendages; heterothallic.

On dung, in humous soil, etc.

Mucor. Saprophytic fungi. Mycelium of one kind, largely penetrating the substratum, without rhizoidiferous stolons. Sporangiophores produced singly (Fig. 3, a-d). Zygospores unprotected. Gametes about equal, heterothallic or at least produced from widely separated parts of the mycelium. (Over one-third of the described Mucoraeceae belong to this genus.)

Key to Species

I.	Spo	rangiophores not	branched.				
	a.	Sporangiophores	not exceeding	ı cm. iı	n length	$\dots M$.	hiemalis
	b.	Sporangiophores	2-15 cm. long.			$\dots M$.	mucedo

- III. Sporangiophores branched in sympodial cymes.

 - c. Sporangiophores straight, not circinate columella spinescent (Fig. 3, f)

M. hiemalis Wehmer. Mycelium a bright gray, sporangia green to yellow-black $50-80\mu$ in diameter. Columella globose when young becoming somewhat elongated. Spores regularly ellipsoidal $4-10\times2.5-5\mu$. Chlamydospores numerous in the substratum, irregular pyriform, barrel shaped, etc. (Fig. 3, k). Zygospores globose $70-100\mu$ black warty; heterothallic.

Common in soil and on organic matter.

M. mucedo (Linne) Brefeld. Sporangiophores erect, rigid simple 2-15 cm. high becoming brown when old. Sporangia spherical (Fig. 3, e) 100-200 μ becoming brown when old, covered with slender crystals. Columella high-cylindrical to globose (Fig. 3, g). Spores twice as long as broad 6-12 by 3-6 μ , smooth weak yellow to colorless. Zygospores globose 90-250 μ in diameter, black, heterothallic.

Common in soil or on organic matter.

M. racemosus Fresenius. Sporangiophores 5-40 mm. long, rigid, irregularly branched (Fig. 3, b) in mass a dirty light yellowish color. Sporangia spherical 20-70μ in diameter, dirty yellow to brownish, the sporangial wall smooth, not dissolving in water, but breaking open easily. Spores hyaline to dirty yellow ellipsoidal to globose 4-8x4-10μ smooth. Zygospores globose 70-85μ in diameter, brown warty, heterothallic. Chlamydospores abundant even on the sporangiophores; globose to oblong (Fig. 3, i) 10-20x25-30μ wall smooth, contents containing oil drops.

Common in soil and on organic matter.

M. botryoides Lendner. Sporangiophores as shown in Fig. 3, c, 1.5 cm. in height. Sporangia globose, clear gray, the walls diffluent in water. Terminal sporangium 80μ in diameter; columella globose (Fig. 3, h). Spores globose 4-10 μ in diameter, uneven. Chlamydospores lemon shaped $16-22\times10-16\mu$.

Fairly common in soil.

M. circinelloides Van Tieghem. Sporangiophores sympodially branched (Fig. 3, d), branches 5-6 often appearing as sessile, brownish. Sporangia globose variable in size, those of the larger having diffluent walls, while those of the smaller are persistent. Spores $3-4x5-6\mu$ smooth, weak gray in mass. Zygospores globose red-brown with long thorn-like pointed warts that are streaked longitudinally; heterothallic.

Fairly common in soil and on organic matter.

M. plumbeus Bonorden. Sporangiophores rigid, erect, straight, up to 1 cm. high, branched, all branches ending in sporangia. Sporangia globose, small, up to 100μ in diameter, dark brown to black at maturity, finely spiny, membrane dissolving leaving a basal collar. Columella long cylindrical to pyriform with one or more spines on the summit (Fig. 3, f). Spores globose, yellowish brown with irregular folds. Chlamydospores as in M. racemosus.

Fairly common in soil and on organic matter.

Zygorrhynchus—Separated from mucor by the gametes, which are unequal and arise comparatively close together, almost invariably originating from a single aerial hypha (Homothallic).

Z. vuilleminii Namyslowski. Sporangiophores flexible, septate variable in length. Sporangia globose 30-70µ in diameter covered

with needles of calcium oxalate, membrane diffluent only when old. Columella broader than high (Fig. 4, b). Spores $2x4\mu$ (Fig. 4, d). Chlamydospores $40x14\mu$, usually in chains. Zygospores brownish, covered with tubercles $2-3\mu$ high (Fig. 4, a and c). Azygospores frequent, smaller than zygospores.

Z. moelleri Vuillimin. Differs only in size of spores. Spores $3x5-7\mu$.

Both species are found in soil and on organic matter.

Phycomyces—Saprophytic fungi; sporangiophores simple arising singly metallic-green or olive, terminated by a large sporangium; sporangia many spored, the membrane dissolving; columella pear shaped (Fig. 5, c). Zygospores dark brown, protected by dichotomously branched spiny outgrowths from the suspensors.

P. nitens. Kunze. Sporangiophores 7-30 cm. high (Fig. 5, a). Sporangia about 1 mm. in diameter; spores ellipsoid 16-30x8-15 μ (Fig. 5, b). Zygospores round, 300 μ thick (Fig. 5, d), typically heterothallic.

On oily or decaying organic matter, especially on old bones or other oily matter.

Spinellus—Differs from Mucor only in the mycelium being of two kinds. (It is often included in the genus Mucor.) The mycelium in the substratum is flexuous, smooth and colorless, that in the air brown, and covered with spines. Sporangiophores and zygospores are both produced only on the aerial mycelia (Fig. 6).

S. fusiger (Lk.) Van Tieghem. Sporangiophores single, unbranched, rigid below bulbous inflated (Fig. 6, e), blue-gray to chocolate-brown at maturity, 0.1-6 cm. high; sporangia black at maturity 180-300 μ in diameter, with a sub-conical columella (Fig. 6, b). Spores spindle shaped, brown 30-40x9-12 μ (Fig. 6, d). Zygospores dark brown 180-400 μ thick; homothallic (Fig. 6, c).

Common, parasitic on agarics.

Sporodinia (Syzygites)—Vegetative filaments delicate, penetrating the substratum. Aerial filaments dichotomously branched producing sporangia and zygospores. Sporangia spherical with hemispherical columella. Zygospores spherical, smooth, homothallic (Fig. 7; Plate V).

S. grandis Lk. Sporangiophores repeatedly dichotomous, septate (Fig. 7, a and b). Sporangia pale red or orange when young, at maturity brownish or blackish brown. Spores round or ellipsoid II-40 μ . Zygospore mycelium brown, the ends tapering (Fig. 7, c).

Common in nature upon decaying Boleti and other large, fleshy fungi, but can readily be grown upon bread or other organic media.

II. TRIBE PILOBOLEAE

Sporangia of one kind only with membrane for the major part solid, persistent, of a very dark blackish color, or swelling only toward the base. Sometimes the sporangium dissolves, leaving the columella, while more often it is forcibly thrown off with the columella and opens only after swelling of the membrane. Zygospores naked (Fig. 8, e).

PILOBOLUS (=Hydrogera)—This is the only genus found in our flora. In this genus the sporangiophore is swollen above and the sporangium thrown off.

Key to Species

2. Swelling at top of sporangiophore almost sphericalP. roridus

P. crystalinus Tode. Sporangiophores 5-10 mm. long (Fig. 8, a), columella conical (Fig. 8, b). Spores 5-10x3-6 μ , colorless (greenish yellow in mass).

On dung (usually appears on horse dung left for a few days under a bell jar).

P. roridus Persoon. Sporangiophores 1-2 cm. high (Fig. 8, d), columella rounded, short. Spores 8-6x3-4 μ , colorless (pale yellow in mass).

On dung.

P. oedipus Mont. Sporangiophores 1-3 mm. high (Fig. 8, c), contents orange red, columella conical, reaching almost to the summit of the sporangium. Spores round, 10-14 μ , orange.

On excrement of animals, on mud, on decaying algae.

III. TRIBE THAMNIDIAE

Sporangia as in the Mucoreae, but of two kinds: the one many spored, with membrane that dissolves, leaving only a naked columella; the other (sporangioles) few spored with a persistent membrane, often without columella. The sporangioles are produced on the ends of branched sporangiophores, which are formed at regular intervals on the principal sporangiophores. Zygospores as for Mucoreae.

KEY TO GENERA

- 1. Primary sporangia with, sporangioles without, columella... Thamnidium

THAMNIDIUM—Sporangiophores erect, principal sporangia terminal on the main branches, with columella; sporangioles on side branches, without columella.

T. elegans Link. Sporangiophores (Fig. 9, a) 0.5-3 cm., occasionally 6 cm. high, the branching very variable. Sporangia 100-200 μ in diameter, white, with large columella, many spored (Fig. 9, d). Sporangioles globose, small, white 8-16 μ in diameter, mostly 4 spored (Fig. 9, b). Spores 8-10x6-8 μ smooth, weak gray brown. Zygospores globose, black, warty (Fig. 9, e).

On dung, in soil, on decaying plant parts, etc.

T. amoenum (Preuss) Schroet. Differs from T. elegans in that the sporangioles are produced on the coiled tips of lateral branches (Fig. 9, c). Sporangia are brownish, with a large eggshaped columella. Spores 6-8x4-6 μ .

On decaying wood, dung, etc.

DICRANOPHORA (Fig. 10)—This genus is rarely found, but is mentioned because of its having gametes entirely unequal, homothallic (Fig. 10, a), and because of its peculiar sporangiophores (Fig. 10, a-b).

IV. TRIBE MORTIERELLEAE

Sporangia without a columella (Fig. 11, a-b), membrane dissolving readily. The zygospores surrounded by a densely interwoven mass of hyphae which grow from the suspensors, and from the branches from which they arise (exterior Fig. 11, d, section Fig. 11, e).

Only one genus and one species will be mentioned, Mortierella polycephala, Coemans. Mycelium much branched and stolon-like, fusing with neighboring hyphae to form a network, septate when old. Sporangiophores erect 250μ high, in groups of 5-20, swollen at the base, tapering to the top, terminating in a large sporangium, and on the upper portion bearing 2-10 short branches terminating in small sporangia. Sporangia round, white 4-20 spored, spores $10-12\mu$, with a large, glistening oil drop.

On dung, decaying fungi, etc.

V. TRIBE CHAETOCLADIAE

Sporangia and "conidia" both produced or only "conidia" (the conidia are to be regarded as reduced one celled sporangia). "Conidia" formed singly (not in chains) upon the ends of the usually much-branched conidiophores. Zygospores naked. Chlamydospores, round intercalary.

CHAETOCLADIUM—This is the only genus commonly met with. Usually parasitic upon other Mucoraceae, occasionaly saprophytic. Mycelium thin, colorless, forming clusters of short, thick haustoria at point of attachment with the hyphae of the host (Fig. 12, d). Conidiophores creeping, verticillately branched. Conidia produced on the swollen middle portion of the branches, the ends of which are sterile (Fig. 12, b, c).

C. jonesii (B. and B.) Fresenius. Conidia round 6.5-10 μ , singly, colorless, but blue in mass.

On Mucoraceae (partially saprophytic).

C. brefeldii Van Tiegh and Le Mon. Conidia globose or globose elliptical, smooth, colorless 2-5 μ .

Parasitic on Mucor mucedo and Rhizopus nigricans.

VI. TRIBE CEPHALIDEAE

"Conidia" seemingly produced in chains on the ends of simple or branched conidiophores. (Really produced in a row in an elongated sporangium which soon disappears.) Zygospores naked.

KEY TO GENERA.

PIPTOCEPHALIS—Parasitic on other Mucoraceae by means of filiform haustoria (Fig. 13, b). "Conidiophores" repeatedly dichotomously branched (Fig. 13, a), erect, septate, brownish with age, not swollen at tip. "Conidia" cylindrical or spherical in radial chains clustered on the ends of the branches. Zygospores spherical, naked (Fig. 13, c).

P. tieghamiana Matruchot. Conidia spindle-shaped to cylindri-

cal, 4-5x2-2.5μ.

Parasitic on Rhizopus nigricans (rare).

Syncephalis—Parasitic on other Mucoraceae (or saprophytic). Mycelium of very slender branching and anastomosing filaments, producing numerous clusters of rhizoids which penetrate the host (Fig. 14, i). "Conidiophores," stout, erect, mostly unbranched, enlarged above; "conidia" cylindrical to fusiform, in many radiating chains clustered on the enlarged summit of the conidiophore. Zygospore spherical, naked, rarely produced as a lateral outgrowth from the fertilized cell. Many species are apt to be found, but none very common, so only a key to species will be given.

Key to Species

Key to Species					
I. "Conidiophores" erect (Fig. 14, a).					
I. "Conidia" produced directly upon the enlarged end of the					
"Conidiophore" (Fig. 14, g).					
a. Spores 8-9x3-4 ^{\mu}					
"Conidiophores" 420-7204 high					
b. Spores 20-27x7-11# "Conidiophores"					
very slender 400-475\mu high					
2. "Conidia" produced upon short branches of the enlarged end of the					
conidiophore (Fig. 14, d, e, f).					
a. Spores cylindric 60-80x5-64					
"Conidiophores" 2-3mm. high					
b. Spores rectangular 13-16x7-8µ					
"Conidiophores" 300-3504 high					
c. Spores cylindric 8-10x6\mu					
"Conidiophores" 120-150µ high					
d. Spores cylindric 5-6x3\mu					
"Conidiophores" o.5mm. high					
II. Conidiophores incurved (Fig. 14, h and c).					
a) Spores 10-12x4-5\(mu\)					
"Conidiophores" 170-200µ high (Fig. 14, c)					
b) Spores 7-8x3-4µ					
"Conidiophores" 100-1204 high (Fig. 14, b)					

- S. sphaerica Van Tieghem-On horse dung with Mucoraceae.
- S. tenuis Thaxter-On sphagnum.
- S. cordata Van Tieghem-On dung.
- S. pycnosperma Thaxter-On dung of mice and sheep.
- S. nodosa Van Tieghem-Parasitic on Mucoraceae.
- S. depressa Van Tieghem-On horse dung.
- S. cornu Van Tieghem and Le Mon.—Parasitic on Mucoraceae.
- S. reflexa Van Tieghem-On dung.

5. Literature

Aside from the general treatment of the Mucoraceae in such publications as Engler and Prantl's "Die Natürlichen Pflanzenfamilien," Saccardo's "Sylloge Fungorum," Rabenhorst's "Kryptogamen Flora," the literature is very greatly scattered. Only a few of the more easily obtained publications in English will be listed.

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